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Dated 15 NOV 1999

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Request for the grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

1. Your reference — REP05956GB

2. Patent application number *(The Patent Office will fill in this part)* — 9827814.6

17 DEC 1998

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Microscience Limited
67-68 Jermyn Street
London
SW1Y 6NY
United Kingdom

Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation — GB

4. Title of the invention — VIRULENCE GENE AND PROTEIN, AND THEIR USE

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

GILL JENNINGS & EVERY

Broadgate House
7 Eldon Street
London
EC2M 7LH

Patents ADP number *(if you know it)* — 745002

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor

b) there is an inventor who is not named as an applicant, or

c) any named applicant is a corporate body.

See note (d)) — YES

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	4
Claim(s)	1
Abstract	
Drawing(s)	

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. For the Applicant
Gill Jennings & Every

I/We request the grant of a patent on the basis of this application.

Signature

Date
17 December 1998

12. Name and daytime telephone number of person to contact in the United Kingdom

PERRY, Robert Edward
0171 377 1377

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Notes

a) If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.

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d) If you have answered 'Yes' Patents Form 7/77 will need to be filed.

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VIRULENCE GENE AND PROTEIN, AND THEIR USE

Field of the Invention

This invention relates to a virulence gene and protein, and their use. More particularly, it relates to their use in therapy and in screening for drugs.

Background of the Invention

E. coli is an organism that is implicated in septicaemia, meningitis, urinary tract infection, wound infection, abscess formation, peritonitis and cholangitis. It would be desirable to provide means for treating or preventing conditions caused by *E. coli*, e.g. by immunisation.

The *yggN* gene of *E. coli* K12 is known; see EMBL and Genbank accession numbers P46143, AE000378 and U28377. *YggN* encodes a 26.4 kD protein of unknown function. The *yggN* gene of *E. coli* K12 is located at minute 68.7, adjacent to the known gene *ansB*, which encodes L-asparaginase II.

Summary of the Invention

The present invention is based on the discovery of a virulence gene in *E. coli* K1, that has homology with the *yggN* gene of *E. coli* K12. Accordingly, the present invention provides:

The therapeutic use of a peptide encoded by the *yggN* gene in *E. coli* K1 or K12, or a homologue thereof in a Gram-negative bacterium, or a functional fragment thereof, e.g. a peptide comprising all or part of the 134-member amino acid sequence defined below;

a host transformed to express the peptide or modified to disrupt expression of the gene;

a vaccine comprising such a peptide or the means for its expression, or an attenuated vaccine in which the virulence gene is disrupted;

the use of the peptide or corresponding polynucleotide as a target for screening potentially useful drugs, especially anti-microbials, or as a diagnostic agent in the detection of virulence, e.g. for testing for the presence of virulent coliforms in livestock.

Description of the Invention

The virulence gene in *E. coli* K1 was identified by using signature-tagged mutagenesis (STM) to screen an *E. coli* K1 mini-Tn*5* mutant bank for attenuated mutants, in a mouse model of systemic infection. Bacteria containing a mini-Tn*5* insertion within the virulence gene failed to be recovered from mice inoculated with a mixed population of mutants, and are therefore likely to be attenuated.

The cloned *E. coli* K1 nucleotide sequence immediately following the mini-Tn*5* insertion is as follows:

Length: 402 nucleotides

```
   1  CGTAGCACCC TGCCGTGGAT TGATGAAGGC GCGAAAAGCC GCGTCGAGAA
  51  AGCTCGTATT GCGCTGGATA AAATTATCGT TCAGGAGATG GGCGAAAGCA
 101  GCAAAATGCG CAGCCGTCTG ACCAAACTTG ATGCGCAGCT GAAAGAGCAG
 151  ATGAACCGCA TTATCGAAAC GCGCAGCGAT GGCCTGACGT TTCACTATAA
 201  AGCCATTGAT CAGGTTCGTG CCGAAGGCCA GCAATTAGTG AATCAGGCAA
 251  TGGGCGGAAT TTTACAGGAC AGCATTAATG AAATGGGCGC GAAAGCGGTG
 301  CTGAAAAGCG GCGGTAACCC ATTACAGAAC GTGCTGGGAA GCCTGGGCGG
 351  CCTGCAATCC TTAATCCAAC CCGAGTGGAA AAAGCAGGAA AAAGATTTCC
 401  AG
```

A translation of this sequence is as follows:

Length: 134 amino acids

```
   1  RSTLPWIDEG AKSRVEKARI ALDKIIVQEM GESSKMRSRL TKLDAQLKEQ
  51  MNRIIETRSD GLTFHYKAID QVRAEGQQLV NQAMGGILQD SINEMGAKAV
 101  LKSGGNPLQN VLGSLGGLQS LIQPEWKKQE KDFQ
```

These sequences show 96% identity to the *yggN* gene of *E. coli* K12, and 98.5% identity to amino acids 81 to 214 of the latter.

This demonstrates that the disrupted gene is at least partially identical to the *yggN* gene of *E. coli* K12.

GCG bestfit analysis at the amino acid level is as follows

```
          .         .         .         .         .
  1 RSTLPWIDEGAKSRVEKARIALDKIIVQEMGESSKMRSRLTKLDAQLKEQ 50
    ||||||||||||||||||||||||||||||||||||||||||||||||||
 81 RSTLPWIDEGAKSRVEKARIALDKIIVQEMGESSKMRSRLTKLDAQLKEQ 130
          .         .         .         .         .
 51 MNRIIETRSDGLTFHYKAIDQVRAEGQQLVNQAMGGILQDSINEMGAKAV 100
    ||||||||||||||||||||||||||||||||||||||||||||||||||
131 MNRIIETRSDGLTFHYKAIDQVRAEGQQLVNQAMGGILQDSINEMGAKAV 180
          .         .         .
101 LKSGGNPLQNVLGSLGGLQSLIQPEWKKQEKDFQ 134
    |||||||||||||||||| || ||||||||||
181 LKSGGNPLQNVLGSLGGLQSSIQTEWKKQEKDFQ 214
```

The 134 amino acid sequence shows no strong homology to any other sequences currently in the EMBL or GenBank databases.

The novel gene has been tested for attenuation of virulence, using mixed infections, in a murine model of systemic infection (Achtman *et al.*, 1983, Infection and Immunity, vol 39, pages 315-335), and shown to be attenuated with a competitive index (CI) of 0.43 (mean CI from three mice).

The *E. coli* K1 *yggN* gene is likely to be useful both in generating attenuated vaccine strains and as a target for antimicrobials

For the purposes of this invention, the appropriate degree of homology is typically at least 50%, preferably at least 60% or 70%, and more preferably at least 80% or 90% (at the amino acid or nucleotide level).

It is evident that *E. coli* K1 strains containing disruptions of the invention are attenuated. The products of the invention may be immunogenic. They are therefore useful in therapy, and more particularly as a prophylactic, in a vaccine.

The protein may be purified. It may be sequenced. The corresponding full-length gene can thus be identified. It can thus be prepared by recombinant technology, by expression in a suitable host. Active fragments and

homologues can be identified. Vaccine compositions, including attenuated vaccines, can be formulated, with carriers and adjuvants as necessary or desired, and used in therapy, to provide an effective immunisation against *E. coli*. In some cases, antibody may be used, for passive immunisation. All these procedures are known to those of ordinary skill in the art, and do not affect the nature of the invention that has been made.

CLAIMS

1. A peptide encoded by the *yggN* gene *E. coli* K1 or K12, or a homologue thereof in a gram-negative bacterium, or a functional fragment thereof, for therapeutic use.

2. A peptide according to claim 1, comprising the 134-member amino acid sequence defined herein.

3. A polynucleotide encoding a peptide according to claim 1 or claim 2, for therapeutic use.

4. A host transformed to express a peptide according to claim 1 or claim 2.

5. A vaccine comprising a peptide according to claim 1 or claim 2, or the means for its expression.

6. A vaccine comprising a microorganism having a virulence gene deletion, wherein the gene encodes a peptide according to claim 1 or claim 2.

7. Use of a product according to any of claims 1 to 4, for screening potential drugs or for the detection of virulence.

8. Use of a product according to any of claims 1 to 4, for the manufacture of a medicament for use in the treatment or prevention of a condition associated with infection by *E. coli*.

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Form 237/77: 9.11.99

AGENT: Gill Jennings & Every